

EMBRYOLOGY OF *JUBAEOPSIS CAFFRA* BECC.: 1 MICROSPORANGIUM, MICROSPOROGENESIS AND MICROGAMETOGENESIS

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ABSTRACT

Development of the anther wall is of the Basic type. Microsporogenesis is simultaneous and results in the formation of tetrahedral tetrads. Pollen grains, which have a smooth exine, are monocolpate and at the time of anther dehiscence, the grain is bi-nucleate.

During microgametogenesis, both the vegetative and the generative nuclei migrate into the pollen tube where mitosis of the latter nucleus results in the formation of two male gametes. The vegetative nucleus degenerates progressively from the initial stages of germination of the pollen grain and finally disappears just prior to the formation of the male gametes. *J. caffra* is apparently a polyploid species.

UITTREKSEL

EMBRIOLOGIE VAN *JUBAEOPSIS CAFFRA* BECC.: 1. MIKROSPORANGIUM, MIKROSPOROGENESE EN MIKROGAMETOGENESE

Ontwikkeling van die helmknopwand is van die Basiese tipe. Mikrosporogenese is gelyktydig en lei tot die vorming van tetraëdriese tetrades. Stuifmeelkorrels, wat 'n gladde eksien besit, is monokolpaat en is met oopspringing van die helmknop, tweekernig.

Tydens mikrogametogenese beweeg beide die vegetatiewe en die generatiewe kerne in die stuifmeelbuis in waar mitose van die laasgenoemde kern lei tot die ontstaan van twee manlike gamete. Die vegetatiewe kern degenerereer progressief vanaf die begin stadia van ontkieming van die stuifmeelkorrel totdat dit net voor die vorming van die manlike gamete heeltemal verdwyn. *J. caffra* is waarskynlik 'n poliploïede spesies.

INTRODUCTION

Jubaeopsis caffra Becc. is the only palm species which is endemic to the Republic of South Africa and it is confined to two extremely small localities in Pondoland on the east coast, viz. the estuaries of the Mtentu and Msikaba rivers (Robertson & Visagie, 1975).

Considering the scarcity of suitable plant material and the problems involved in obtaining it, it is not surprising that no data relating to the embryology of this species have been recorded. It is strange though that so little is known about the embryology of the Palmae as a whole, especially when the size of this family is taken into account.

The structure of the mature anther wall is remarkably well-known for palms in general (Davis, 1966) but no information on the development of the wall is available. Usually the wall consists of a persistent epidermis, an endothecium with fibrous thickenings, two to six ephemeral middle layers and a glandular

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tapetum (Davis, 1966). The tapetum cells are uni-nucleate in *Cocos nucifera* (Juliano & Quisumbing, 1931), *Borassus flabellifer* L. (Venkato Rao, 1955a) and *Hyphaene indica* (Mahabale & Chennaveeraiah, 1957) while in *Areca* (Venkato Rao, 1955a) *Pritchardia* (Venkato Rao, 1955b) *Licuala*, *Livistona* (Venkato Rao, 1956a), *Sabal* (Venkato Rao, 1956b) and *Arecastrum roman-zoffianum* (Venkato Rao, 1958) they are bi- or multinucleate.

With regard to microsporogenesis, simultaneous division of the microspore mother cells occur in the majority of genera and species studied viz. *Chamaedorea*, *Areca triandra*, *Caryota*, *Pteriospermum* (Schnarf, 1931, cited by Mahabale & Chennaveeraiah, 1957), *Cocos nucifera* (Juliano & Quisumbing, 1931), *Borassus*, *Areca* (Venkato Rao, 1955a) *Licuala*, *Livistona* (Venkato Rao, 1956a), *Trachycarpus*, *Sabal* (Venkato Rao, 1956b), *Hyphaene indica* (Mahabale & Chennaveeraiah, 1957), *Caryota*, *Chrysalidocarpus* and *Arecastrum roman-zoffianum* (Venkato Rao, 1958). Successive cytokinesis is apparently restricted to *Nypa fruticans* and *Pinanga disticha* (Davis, 1966).

The palm pollen grain is binucleate (Brewbaker, 1967) and the mitotic division of the generative nucleus takes place in the pollen tube after the grain has germinated (Read, 1963, 1964). The destiny of the vegetative nucleus is uncertain. Eames (1961) states that the tube nucleus, as it is often called, behaves in different ways and may or may not enter the pollen tube. The activities of this nucleus during microgametogenesis of the palm has not yet been described.

MATERIAL AND METHODS

The material for the study of microsporogenesis and the structure of the anther wall was collected during 1973/74 from inflorescences at various stages of development from a 43 year old tree in St. George's Park, Port Elizabeth. Male flowers were fixed in Craf II (Sass, 1958), dehydrated in an ethyl alcohol/tertiary butyl alcohol (TBA) series, embedded in paraffin wax (55°C) and sectioned at 10 μ m on a rotary microtome as prescribed by Brooks, Bradley and Anderson (1950). The sections were stained in safranin/fast green (Holtzhausen, 1972).

Prior to studying microgametogenesis, it was necessary to germinate pollen grains of *J. caffra*. Although various methods were used for this, the most successful method proved to be the coated-slide technique (Conger, 1953 cited by Sharma & Sharma, 1972). Optimum results were obtained with ten per cent sucrose and 0.01 per cent boric acid in agar. To ensure that a large number of metaphase figures would be obtained, 0.05 per cent colchicine was added to the medium. Pollen from flowers which had been collected a day earlier was sprinkled onto the coated slides and incubated in the light at 25°C in a plastic container which had been lined with moist filter paper. By fixing and staining at various times, it was established that the most metaphase figures were ob-

tained after 18–20 hours of incubation.

After incubation, the slides were fixed in acetic acid/alcohol (1:3) for 12 hours. Thereafter, dehydration by means of a TBA series and staining with propiono-carmin (Vasil, 1963) took place. The stained slides were made permanent by means of the alcohol vapour method (Brooks *et al.*, 1950).

The scanning electron microscopic study was conducted on a Jeol JSM-U3 microscope with fresh pollen, which had been coated with palladium gold (Robbertse, 1974).

RESULTS AND DISCUSSION

Each anther is composed of four microsporangia (Fig. 1). This condition is maintained throughout the lifetime of the anther and is still evident at the time of dehiscence of the anther and, although the wall separating the two sporangia in each lobe becomes crushed, it does not break down completely (Fig. 1).



FIG. 1.
A transverse section through the anther of *J. caffra*.

Initially the anther comprises the connective plus four lobes of undifferentiated homogeneous cells. As soon as the tip of the inner bract enveloping the inflorescence becomes visible in the axil of the leaf, periclinal divisions occur in the hypodermal cells of the anther. This division results in the formation of two cell layers *viz.* an outer one which constitutes the primary parietal layer and an inner cell layer, the primary sporogenous layer.

The latter layer undergoes mitotic divisions in both anticlinal and periclinal directions. Thereafter the cells enlarge and their nucleoli become conspicuous (Fig. 2A). These cells constitute the microspore mother cells.

The cells of the primary parietal layer divide periclinally to produce the two secondary parietal layers. The cells of these two layers in turn enlarge and divide periclinally as well (Fig. 2A) to produce four cell layers between the epidermis of the anther or microsporangium and the sporogenous tissue (Fig. 2B). Of these four layers the outermost one will differentiate to form the endothecium; the innermost cell layer *i.e.* adjacent to the sporogenous cells, develops into the tapetum and the two remaining layers constitute the middle layers of the anther wall.

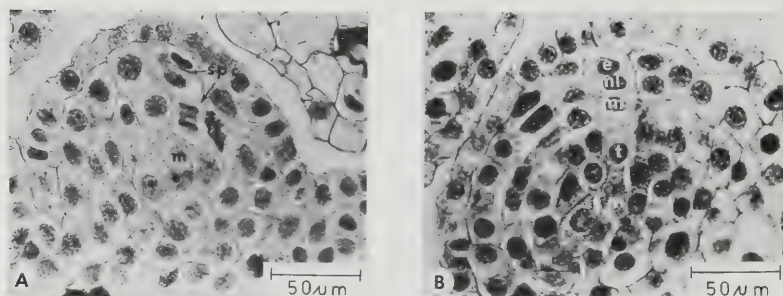


FIG. 2.

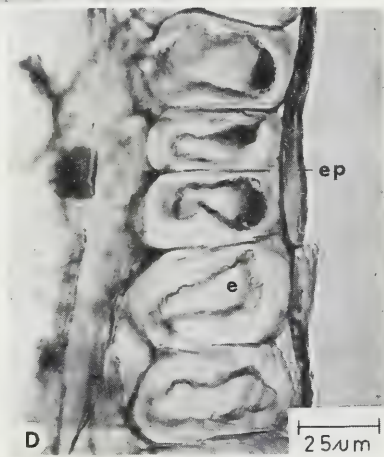
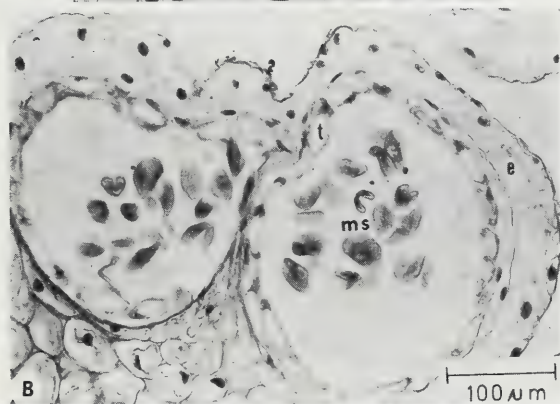
Transverse sections of the lobe of a young anther.

- A. Periclinal divisions of the two secondary parietal layers (spc.).
 B. The four cell layers constituting the anther wall *viz.* endothecium (e), two middle layers (ml) and the tapetum (t).
 Also visible in both figures are the microspore mother cells (m).

In the anther wall of *J. caffra*, two middle layers occur and each of the two secondary parietal layers are involved in their formation. It is concluded thus that the Basic type (Davis, 1966) of anther wall formation is found in this species. According to Davis this type of development represents a primitive condition. Some difference of opinion concerning the various components of the anther wall, especially in respect of the tapetum, is to be found in the literature. According to Eames (1961), the primary sporogenous cells divide periclinally to give rise to both the tapetal cells and the sporogenous cells. This is in direct contrast to the reports by Maheshwari (1950) and Davis (1966) both of whom state that the tapetum has a non-sporogenous origin and that it is derived solely from the

FIG. 3.

- A-C Transverse sections of microsporangia showing the development of the various cell layers at different stages of development.
 D. Longitudinal section through the wall of a mature anther.
 (e-endothecium; ep-epidermis; m-microspore mother cells; ms-microspores; t-tapetum).



parietal tissue. The above data show clearly that in *J. caffra* the tapetum is derived from the parietal tissue only.

During the early stages of the ontogeny of the anther, the epidermis cells of the anther wall are more or less isodiametric in shape in a transverse section through the anther (Fig. 2A). During subsequent growth, the surface area of the anther increases very rapidly and although anticlinal divisions do occur in the epidermis cells, they cannot keep pace with the increase in volume of the underlying cells. Consequently the epidermis cells become stretched and flattened in a tangential plane (Fig. 3B & C) but the cells are not cast off. The epidermis is therefore of a persistent nature.

The endothecium cells steadily increase in the size until the completion of meiosis (Fig. 3B). At maturity the characteristic "fibrous" thickenings are present as bars across the radial cell walls (Fig. 3C). These bars are visible only in a transverse section through the anther and not in a longitudinal section (Fig. 3D).

Development of the "fibrous" thickenings is not initiated until the microspore tetrads separate into individual pollen grains (Fig. 3B & C). Although the thickenings occur mainly in the endothecium cells, they can also be observed in the cells of the outer middle layer, especially in the vicinity of the connective (Fig. 3C). This phenomenon has been reported in a number of other monocotyledons e.g. *Agave*, *Crinum* and the Zingiberaceae (Davis, 1966). A large number of starch grains are present in the older endothecium cells.

The cells of the two middle layers do not undergo anticlinal divisions and soon become tangentially stretched and radially flattened (Fig. 3B). These two cell layers are ephemeral and become crushed and degenerate sometime prior to the onset of meiosis in the microspore mother cells (Fig. 4A). The outer layer is however relatively more persistent than the inner middle layer.

The tapetum cells remain uninucleate. They form a single layer around the sporogenous tissue and become radially elongated and are initially closely packed against each other with no intercellular spaces between them (Fig. 3A). This condition is short lived though, and as the two middle layers degenerate and the circumference of the sporangium increases the tapetum cells become loosely arranged, but maintain a more or less radial orientation. After meiosis, the radial organisation is no longer evident and the cells of the tapetum remain only as a thin layer around the periphery of the anther loculus (Figs. 3B & C).

At this stage *i.e.* after the formation of the pollen grains, the walls of the tapetum cells are still intact and remain *in situ* and consequently it is concluded that the tapetum of *J. caffra* is of the glandular or secretory type (Maheshwari, 1950). This is in accordance with the condition found in other palms (Davis, 1966) and is the type of tapetum most commonly found in the dicotyledons and advanced or more specialised monocotyledons (Eames, 1961).

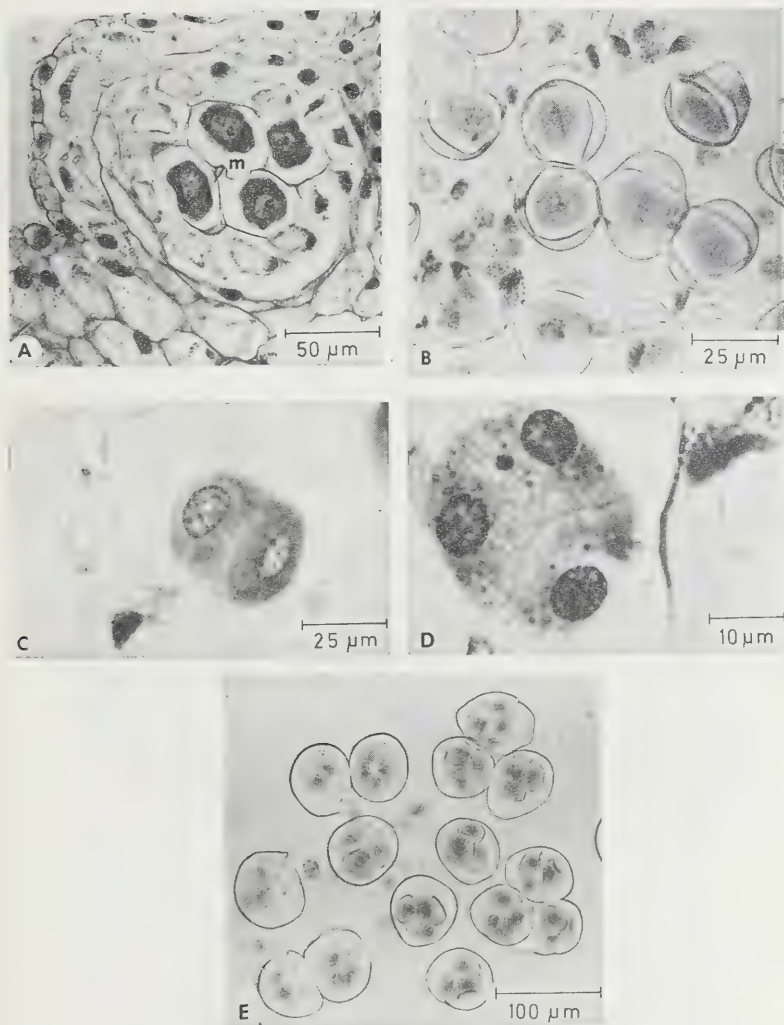


FIG. 4.

Microsporogenesis in *J. caffra*.

- A. Microspore mother cells (m) shortly prior to meiosis.
- B. Prophase I.
- C. Telophase I. Note the absence of a dividing wall.
- D. Telophase II showing the first signs of cell wall formation.
- E. A squash preparation of the tetrahedral microspore tetrads.

Microsporogenesis

The primary sporogenous cells do not function directly as the microspore mother cells but first undergo a limited number of mitotic divisions. These mother cells are very different from the surrounding tissues in that they are large cells with dense cytoplasm, large nuclei and prominent nucleoli (Fig. 4A).

Initially the cell walls between the mother cells are thin (Fig. 4A) but shortly before meiosis, the cell walls become very much thicker (Fig. 4B).

As in most other palms (Davis, 1966) cytokinesis is of the simultaneous type (Maheshwari, 1950) and no cell wall is formed after the first division (Fig. 4C). Wall formation is delayed until after Telophase 2 (Fig. 4D). The actual division results from cell-plate formation.

The microspore tetrads are tetrahedral (Fig. 4E). At the completion of meiosis the tetrad is surrounded by a thick callose wall. This wall however degenerates soon afterwards and the microspores are released into the anther loculi.

Each microspore is initially enclosed in a thin wall which becomes thicker shortly prior to dehiscence of the anther. The exine is relatively thin and smooth without any spines. The pollen grain is monocolpate (Fig. 5) and elongate with a furrow or fold on the distal side. This is in accordance with other palms

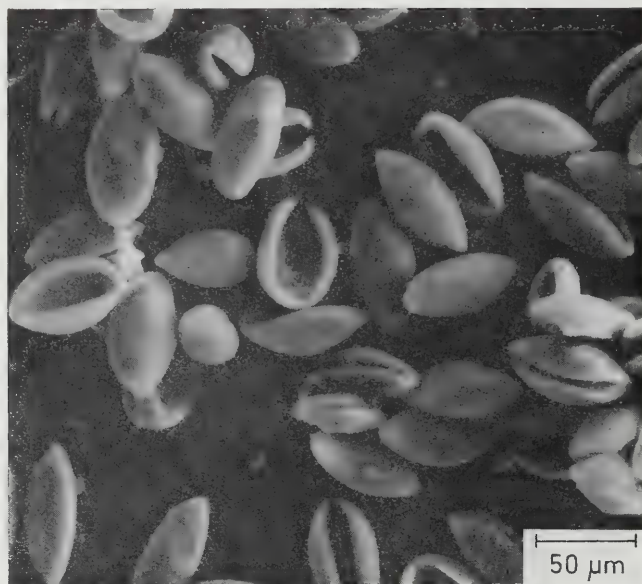


FIG. 5.
Stereoscan micrograph of the pollen of *J. caffra*.

(Parthasarathy, 1970). According to Eames (1961) this type of pollen grain is a primitive feature characteristic of the monocotyledons. The fully imbibed pollen grain is 40 μm in diameter.

Male gametophyte and gametogenesis

Shortly prior to dehiscence of the anther, the large centrally placed nucleus of the microspore divides mitotically and the vegetative and generative nuclei are formed. The vegetative nucleus remains more or less round and centrally situated while the generative nucleus, which is spindle shaped, is confined to the periphery of the microspore (Fig. 6A). The pollen grain is released from the anther in this condition, *i.e.* 2-celled. These findings are in accordance with those of Brewbaker (1967) and Read (1963, 1964) for other palm species.

Germination of the pollen grain is initiated one hour after sowing and by this time the vegetative nucleus has degenerated extensively and is only just visible (Fig. 6B).

In *J. caffra* the vegetative nucleus occasionally disappears completely before the generative cell has entered the tube. In most cases though, the tube nucleus does not disappear completely and faint traces of it remain visible until the late stages of mitosis of the generative nucleus (Figs. 6C & D).

It seems unlikely that a nucleus in such a state of degeneration and disorganisation could play any active role in the growth of the tube. It is consequently suggested that the movement of this nucleus into the pollen tube is purely a passive action and that the tube nucleus is simply borne along by the cytoplasmic flow from the pollen grain into the pollen tube.

The spindle-shaped generative cell moves down into the tube soon after germination of the grain, but its nucleus does not undergo mitosis until at least 20 hours after sowing. Division of the generative cell results in the formation of the two male gametes (Fig. 6E). At this stage the pollen tube is between 600 and 900 μm long.

Chromosome counts

The pollen tube and its haploid nucleus provides material which is well suited for the studying of the chromosome complement of a given plant species. This method has been used with great success by Read (1963, 1964, 1965, 1966; Read & Moore, 1967) specifically on palm material.

According to Read (1966) the subfamily Cocoideae can be divided into two groups on the basis of chromosome complement. The spiny bactroid group *e.g.* *Bactris* and *Acrocomia* with $n=15$ is the first while the second group has $n=16$. The latter group includes *Jubaea chilensis* and *Cocos nucifera*.

It appears from the results of this study that *J. caffra* is a polyploid (Fig. 6F). Owing to the large number and small size of the chromosomes, it was not pos-

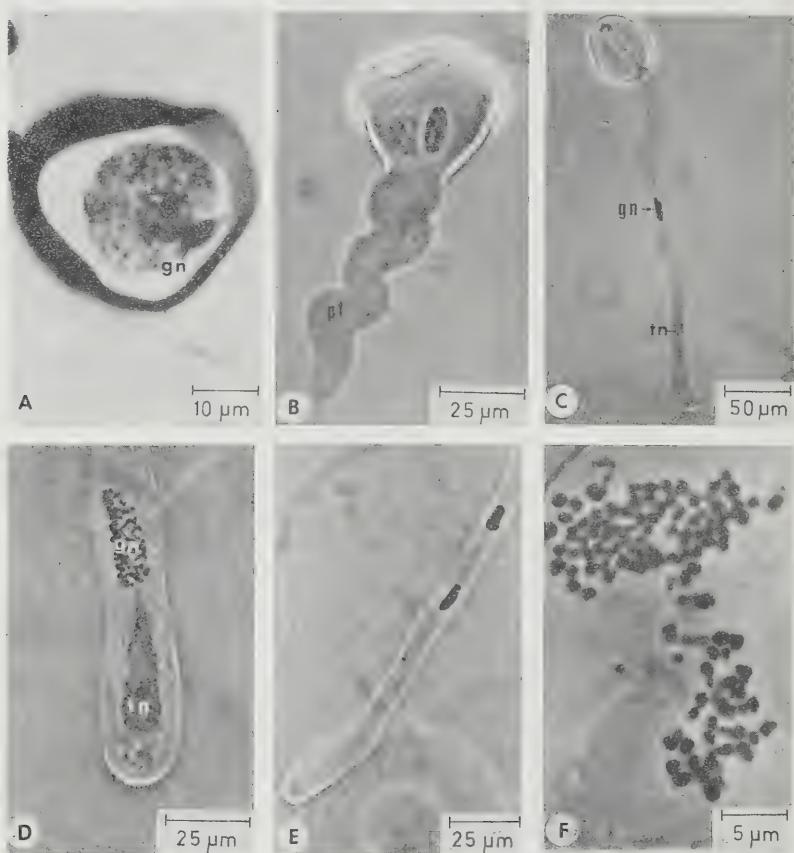


FIG. 6.

Microgametogenesis of *J. caffra*.

- A. Mature pollen grain with vegetative and spindle-shaped generative nuclei.
 - B. Germination one hour after sowing. Both nuclei are still visible.
 - C. Five hours after sowing. Note the remains of the tube nucleus.
 - D. Metaphase during mitosis of generative nucleus.
 - E. Two male gametes in pollen tube 20 hours after sowing. The vegetative nucleus is no longer visible.
 - F. Squash preparation of the pollen tube and generative nucleus during metaphase of mitosis.
- (gn—generative nucleus; pt—pollen tube; tn—tube nucleus; vn—vegetative nucleus).

sible to make a precise count of the chromosome number. However, as shown in Fig. 6F, the haploid complement is in the order of 100 chromosomes. Only

one other polyploid palm has been reported, viz. *Arenga*, a dwarf caryotoid palm from Malaya (Moore & Uhl, 1973) with $n=32$.

CONCLUSION

From the results of this study, it would seem that *J. caffra* exhibits a number of primitive features. Development of the anther wall, for example, is of the Basic type, which according to Davis (1966) is the most primitive type. Unfortunately no information concerning this aspect is available for any other palm.

The pollen of this palm species, like that of the majority of palms, is monocolpate. Pollen of this type is considered by Sowunmi (1967, cited by Moore & Uhl, 1973) as being the most primitive pollen found in the monocotyledons.

While Moore & Uhl (1973) state that the basic haploid chromosome complement of palms is 18, Read (1966) reports that the majority of Cocoid palms have a haploid complement of 16 chromosomes. This suggests that the Cocoid palms in general are more specialised than the primitive Coryphoid and Phoenicoid palms which have $n=18$ chromosomes. *J. caffra* however has a haploid complement of between 80 and 100 chromosomes and is almost certainly a polyploid. In this respect it is unique in that only one other polyploid palm, viz. *Arenga*, is known which has $n=32$.

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